WATER PRIMROSE

by

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Introduction

Water primrose, *Ludwigia grandiflora* (Michx.) Greuter & Burdet ssp. *hexapetala* (Hook. & Arn.) G.L. Nesom & Kartesz (Synonym: *L. hexapetala*), is an aquatic perennial creeper native to South America that invaded freshwater wetlands in California (Okada et al. 2009) and Europe. Its distribution in South America ranges from the tropics to around 40ºS (Munz 1942; Zuloaga 1999). Twenty *Ludwigia* spp. grow in Argentina, many of them sympatric. The species belong to the Oligospermum section, with no clear boundaries and genetic barriers between its members, resulting in extremely variables characteristics. They are all polyploids (x=8) ranging from di- to decaploids chromosomes (Raven and Tai 1979). Hybrids were also recorded among them. The exploration and studies in progress at SABCL aim to discover specific natural enemies against the biotype of *L. g. hexapetala* that invades California.

Plant taxonomy and surveys of *Ludwigia* species south of 34ºS.

Objective: To find stable and reliable characters to identify *L. g. hexapetala*.

Material and Methods

Collection and culture of Ludwigia specimens: A total of 29 sites were surveyed in Buenos Aires Province, some of them were visited twice (Fig. 1). Five stems per site were selected and then cultivated separately in pools in a greenhouse. Pools (1 x 1.2 x 0.4 m) were divided in 15 sectors with a structure of polycarbonate (Fig. 2a) and were filled with 15 cm of soil and completed with water. Specimens were analyzed in spring (active growing) and summer (maturity) recording morphological characters throughout plant phenology (Fig. 2).

Morphological characters: when flowering and fruiting, the following characters were recorded, on stems: pilosity, stipule shape and dimensions; on leaves: length and width, mucro type, number of veins, pilosity; on flowers: petal length and width, pedicel length, style length, bracteole type, sepal; and on fruits: shape, length and diameter, bracteole level, seeds. These data will be analyzed with Principal Component Analysis, adding the data of chromosome number to the comparisons. Same characters were recorded on plants collected in the field to observe growth variations.

Phenology in natural conditions: The variables considered in each site with water primrose were: Site characteristics; water depth; patch size; plant height above the water level; presence of flowers and fruits.

Chromosome counts: Roots in active growth were fixed in 8 hydroxyquinoline and preserved in 3:1 ethanol/acetic acid and stained with Feulgen (Singh 2003). Roots stained where softened

with enzymes, mounted in slides, and analyzed in a compound microscope. A minimum of five cells for each individual in the culture were counted.

Results

Morphological characters: analysis is still in progress; some plants have not bloomed yet and chromosome counts are not completed. Characters such as leaf and stems dimensions will not be analyzed because of the high plasticity of this species; for instance, pilose stems in the field, a key character, change to glabrous growth within a week. Mucro shape of the leaves and persistence of the style base on the fruits could be good characters to distinguish these species.

Phenology in natural conditions: Site characteristics: typical water primrose sites were in the sun, along ditches, small streams and ponds with shallow water (max.0.8m). *Ludwigia g. hexapetala* (80 chromosomes) growing in open water showed in summer a crowded center, with erect plants and stems creeping on the water surface in the edges. Erect stems become dry in winter because of the frost or natural senescence, and the plants re-growth from the creeping stems. This phenological variation was not widespread among *L. peploides* (16 chromosomes) (Table 1) which did not always develop an erect form.

Chromosome counts: so far 255 cells with chromosomes in metaphase were counted; most of the specimens displayed 80 or 16 chromosomes (Table 1; Fig. 3), and a few showed different numbers, multiple of 8, which will be re-checked because of the possibility of hybrids.

Natural enemies

***Liothrips* sp. (Thysanoptera: Phlaeothripinae)**

Taxomomy: These thrips collected in 2009 were identified as a new species by Dr. María Inés Zamar (Inst. Nac. Biol. de Altura, Univ. Nac. de Jujuy) in collaboration with Dr. J.S. Bhatti, India. The description of the new species is in preparation.

Rearing methodology: a- General rearing: Adults of *Liothrips* sp. collected from the field were released on plants growing in a square pot (35x50 cm), with soil and filled with water. Periodically damaged plants were replaced by new ones. b- Rearing methodology for tests: Four different cages were evaluated for easy inspection of the insects, escape prevention, and adequate plant conditions. The container selected consisted in a rectangular translucent recipient (17x10x5cm), with cap and with wet tissue paper in the bottom. Water primrose apical stems (10 cm) were maintained in water picks and changed periodically. The eggs, nymphs or adults were deposited on the leaves with a hair brush and inspected daily.

Biology: The studies were done with specimens collected on *L. g. hexapetala* (80 chromosomes). Eggs collected from general culture were maintained in Petri dishes until the larvae emerged. Five newly emerged larvae were maintained per recipient and observed daily to record the molts. The adults obtained where sexed and distributed in couples to record pre-oviposition time, oviposition period, number of eggs per female, and mortality.

Results: The development time at 25 ºC was 35 days; mortality was higher for the first larval instar (26%), diminishing in the subsequent instars. The main mortality factor was humidity:

either excess or deficit. In addition the behavioral aspects of immatures and adults were recorded.

Specificity test: Host specificity preliminary tests were started with feeding no-choice test. The first two species tested are in different sections: a- *Ludwigia elegans* (Camb.) Hara, Sect. Myrtocarpus; 17 newly emerged nymphs were tested for feeding and 64.7% reject the plant and died; the other 35.3% were missing because they left the stems. Two couples stopped ovipositing when transferred from *L.g. hexapetala* to *L. elegans*; b- *L. lectocarpa* (Nutt.) Hara, Sect. Seminuda. Five newly emerged nymphs tested died without feeding. Control plant was *L. g. hexapetala* on which 15 nymphs survived (87%) with only 2 nymphs missing.

Field trips (Hernández and Sosa)
-Nov.4-5, 2010: To Tres Arroyos, Balcarce and road 2, Buenos Aires Prov.
-March 1-3, 2011. To Tres Arroyos, Balcarce and Tandil, Buenos Aires Prov.

Future plans
-Continue the studies on *L. g. hexapetala*: Correlation between cytology and morphology; seasonal phenology, development in exclusion; etc.
-Continue the evaluation of selected natural enemies, e.g. *Liothrips* sp. and *Tyloderma* spp.
-Increase the number of *Ludwigia* species available at SABCL for choice tests.

Relevant accomplishments
-Morphological and cytological characterization of *Ludwigia g. hexapetala* and *L. peploides*.
-The finding of a new species for science of *Liothrips* sp., which is being described.

References
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Table 1: Plant phenology in Buenos Aires Province sites. (Preliminary analysis)

Season		Sites	Chromosomes	Mean Height
Spring	Nov.	445, 448, 450	16	Stems on water surface (n=3)
Summer	Feb.	044, 045, 046, 047, 047, 049, 050, 051	80	0.39m (n=8)
	March	445, 448, 450	16	Stems on water surface (n=3)
	March	461, 462, 463, 447	80	0.39m (n=4)
Fall	May	166, 167, 169, 171	?	Stems on water surface (n=4)

Fig. 1. Sites surveyed in Buenos Aires Province. Red flags, *L.g. hexapetala* (80 chromosomes). Green dots, *L. peploides* (16 chromosomes). Green-red dots, hybrids? Yellow dots, chromosome counts in progress.

Fig.2. Phenology of *Ludwigia g. hexapetala*. a, in spring; b, in summer; c, in winter leaves in erect stems; d, winter leaves on the water surface.

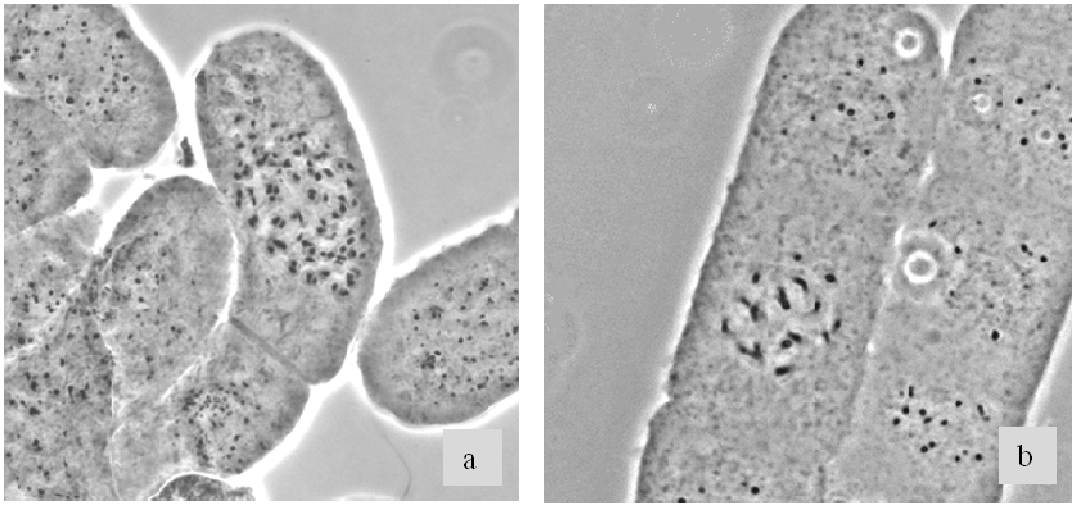

Fig.3. Chromosome numbers: a, *Ludwigia g. hexapetala* (80 chromosomes). b, *L. peploides* (16 chromosomes).